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Atomic basis of the exquisite specificity of phosphate and sulfate transport receptors

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Atomic basis of the exquisite specificity of phosphate and sulfate transport receptors. We have determined, by the method of x-ray crystallography, the 1.7 Å resolution three-dimensional structures of the ligand-bound form of the phosphate receptor as well as the sulfate receptor. These protein structures provide an unprecedented atomic-level understanding of the mechanism governing the exquisite specificity of each receptor. Although they lack amino acid sequence homology, both receptors have very similar three-dimensional structure. The structure consists of two globular domains separated by a deep cleft which contains the ligand-binding site. The bound phosphate and sulfate are totally devoid of water of hydration. The bound phosphate is tightly held in place by 12 hydrogen bonds, 11 with donor and 1 with acceptor groups. The acceptor group (an Asp carboxylate side chain) plays three key roles. It confers specificity by directly recognizing one proton of either the monobasic or dibasic phosphate. It also assists in the recognition of another proton of the monobasic phosphate. Finally, because of charge repulsion, it disallows binding of fully ionized sulfate. The sulfate bound to the sulfate receptor makes seven hydrogen bonds with uncharged polar groups exclusively. The absence of an acceptor group in the binding site of the sulfate receptor is not conducive to phosphate binding.

Transport processes perform a vital function in the life of the cell by maintaining a relative constant environment within the cell and by regulating the entry or exist of various substances necessary for metabolic activity. Often active transport systems exhibit extremely high specificity as shown, for example, by those for phosphate and sulfate, two structurally very similar tetrahedral oxyanions that are the principal sources for phosphorous and sulfur. The specificity of each oxyanion is best exemplified by the binding protein-dependent active transport systems or permeases in gram-negative bacteria [1, 2]; the permease for phosphate is distinct from that for sulfate. The specificity for each oxyanion permease is principally achieved by the presence in the periplasm of a phosphate-binding protein (PBP) and a sulfate-binding protein (SBP), which serve as initial high-affinity receptors [3, 4]. (The designations of PBP or phosphate receptor and SBP or sulfate receptor will be used in this paper.) These receptors bind nutrient and undergo a conformational change (see below) that facilitates interaction with the cytoplasmic membrane components. The membrane-bound components carry out actual nutrient translocation and generate the energy for the active transport. While the receptors are found in the periplasmic space of gram-negative bacteria, we have recently characterized a phosphate receptor anchored on the cell surface of *Mycobacterium tuberculosis* [5]. The specificity of the phosphate permease is also shared by other phosphate transport systems in eukaryotic cells and across brush borders and into mictochondria.

Phosphate receptor and sulfate receptor

To understand ligand specificity at an atomic level, our laboratory has been engaged in the analysis of the structure and function of both receptors principally by x-ray crystallographic technique. The mass of both receptors is about 33 kDa. The dissociation constant of the PBP-phosphate complex in solution at relatively low ionic strength and pH 8.5 is 0.3 μM (P. Ledvina and F.A. Quiocho, unpublished data; [6]). Under identical conditions, the receptor does not bind sulfate (P. Ledvina and F.A. Quiocho, unpublished data). PBP binds both monobasic and dibasic phosphates [6].

SBP also exhibits extremely high specificity, binding sulfate (0.1 μM dissociation constant [7, 8]) but not phosphate [7]. The stringent specificity exhibited by phosphate and sulfate receptors has important biological significance. It means that one nutrient cannot become an inhibitor of transport for the other.

Specificity of receptor-phosphate complex

The atomic structure of the PBP-phosphate complex has been determined by x-ray crystallographic technique at 1.7 Å resolution [9]. As shown in Figure 1, the phosphate is bound in the cleft between the two domains of the receptor. A comparison of this structure with that of the recently determined structure of the ligand free receptor indicates that access to and from the ligand-binding cleft is modulated by a hinge-bending motion between the two domains [N. Yao, P. Ledvina, A. Choudhary, and F.A. Quiocho, unpublished data]. In contrast to the structure of the bound form which shows the two domains being close to each other and engulfing the bound phosphate (Fig. 1), the two domains in ligand-free open structure are farther apart and, hence, the cleft accessible to the solvent.

As shown schematically in Figure 2, the phosphate is completely desolvated and held tightly in place by 12 hydrogen bonds formed with 11 donor and 1 acceptor groups. With the exception of the two donor groups from the guanidinium side chain of Arg 135, the other nine donors are contributed by peptide backbone NH and side chain OH groups. The NH groups are highly polarizable, a feature of value in charge stabilization by way of dipoles. The OH groups could be considered as being 2/3 of a

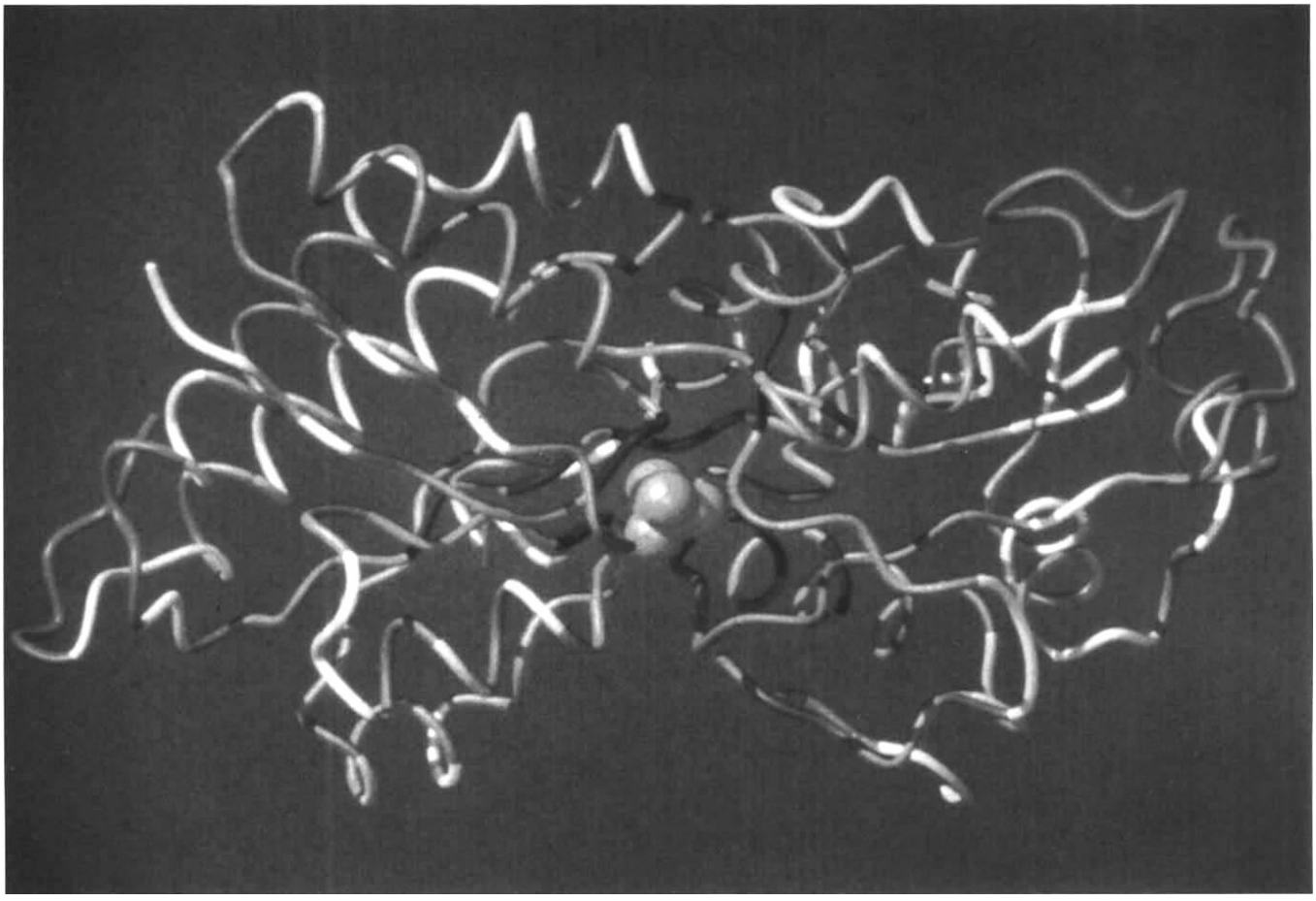

Fig. 1. *Polypeptide backbone trace of the structure of the phosphate receptor or PBP with phosphate (ball) bound in the cleft between the two domains.* This trace is based on the refined 1.7 Å resolution structure of PBP [9].

water molecule. This is apparent by the way the OH groups behave as a bifunctional group, donating and accepting hydrogen bonds (Fig. 2).

Asp 56, the lone charged hydrogen bond acceptor, plays three key roles [9]. By accepting a hydrogen bond from the phosphate, it insures recognition of a proton of the phosphate. The carboxylate group also disallows, by charge repulsion, binding of the sulfate dianion. The fact that the sulfate has no means of satisfying the carboxylate acceptor group may also contribute to the inability of the receptor to bind sulfate. Although less established, the third function of Asp 56 is in facilitating the binding of monobasic phosphate which has one additional donatable. This is possible if the second proton of the monobasic phosphate is on O3. In this way, the second proton is donated to the hydroxyl oxygen of Ser 38 which in turn donates its proton to the carboxylate group of Asp 56 (Fig. 2).

Specificity of receptor-sulfate complex

The atomic features that govern the high specificity of SBP were revealed by the determination of the structure of the receptor-sulfate complex [10, 11]. The structure, originally determined at 2 Å resolution [10, 11], has been recently refined to 1.7 Å resolution [J.S. Sack and F.A. Quiocho, unpublished data]. Although SBP has essentially no sequence similarity with PBP, there is high homology of the structures of both PBP and SBP. Much like in PBP, the sulfate bound to SBP is completely dehydrated and buried in the cleft between the two domains. There are subtle differences in the atomic interactions between SBP-sulfate and PBP-phosphate complexes. The sulfate is bound by seven hydrogen bonds (Fig. 3), five less than the number observed the PBP-phosphate complex. Fully consistent with the SBP specificity, all seven hydrogen bonds are formed with the four sulfate oxygens accepting uncharged donor groups exclusively. The fact that there is no group in position to serve as a hydrogen bond acceptor accounts for the inability of SBP to bind phosphate.

Paradoxically, although the bound sulfate makes five less hydrogen bonds than the phosphate (Figs. 2 and 3), the affinity of the SBP-sulfate complex is only threefold tighter than that of the PBP-phosphate complex (see above). Moreover, while one salt link (with Arg 135) is associated with the bound phosphate, none is formed with the bound sulfate. However, not all of the positive charge from the guanidinium of Arg 135 is neutralized by this salt link because the guanidinium further shares similar ionic and bidentate hydrogen-bonding interactions with the carboxylate of Asp 137 (Fig. 2).

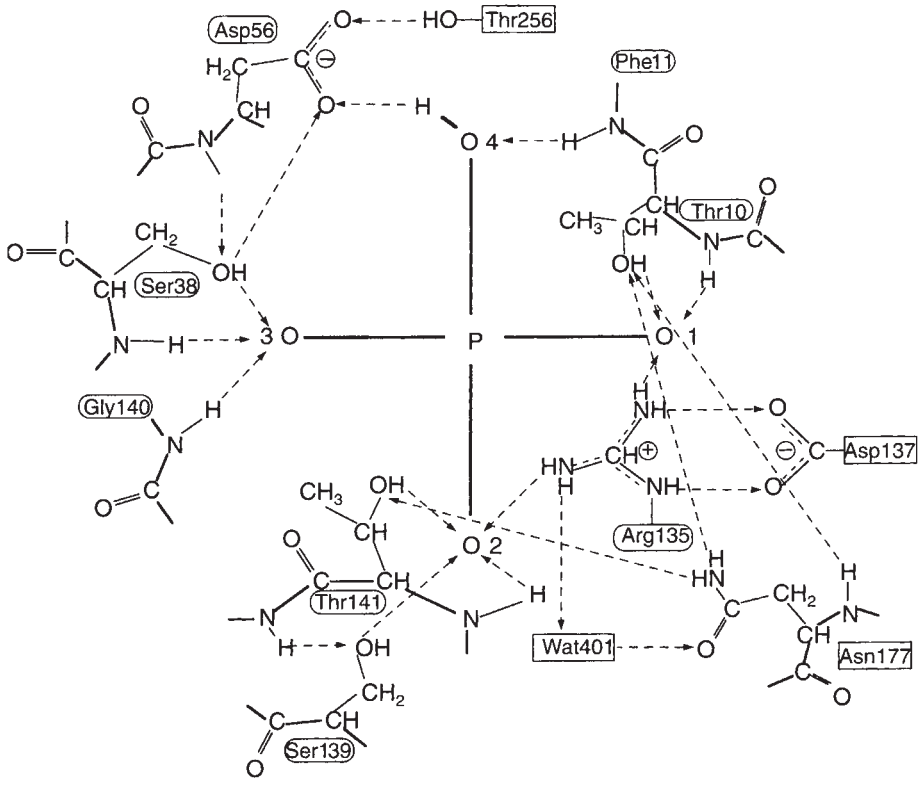

Fig. 2. *Schematic diagram of the hydrogen-bonding interactions between PBP and phosphate.* Adapted from [9].

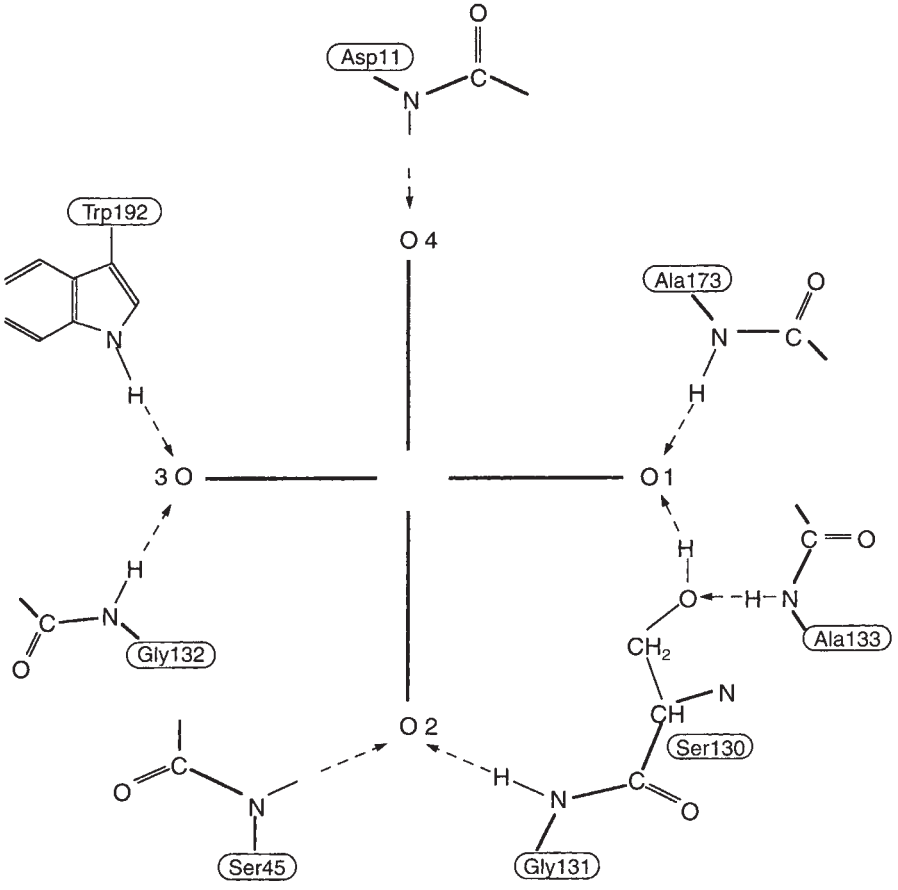

Fig. 3. *Schematic diagram of the hydrogen-bonding interactions between SBP and sulfate.* This diagram is based on the 1.7 Å resolution refined structure of the SBP-sulfate complex (J.S. Sack and F.A. Quiocho, unpublished data; [10, 11]).

Charge stabilization by local dipoles

A finding of paramount importance and wide implication arising from our atomic structure analysis is how are the isolated charges on both phosphate and sulfate oxyanions bound to the receptors stabilized or neutralized. The determination of the SBP-sulfate complex led us to propose that local dipoles, including neutral hydrogen-bonding groups, surrounding the sulfate are almost entirely responsible in stabilization of isolated charges [10, 11]. The importance of this process in charge stabilization in other systems was soon realized [12]. Although the possibility of helix macrodipole contributing to this process was entertained [9], it is not supported by the results of computational and experimental studies [13, 14]. On the other hand, these results confirmed the dominant role of local dipoles from the first or last turn of the helix in charge stabilization. Indeed, the helix terminal ends, combined with other backbone peptide dipolar groups preceding or following them, are a rich source of local dipoles for binding and charge stabilization [14].

In conclusion, although phosphate and sulfate have very similar structures, the presence of proton(s) on the phosphate, a weak acid, or the absence of proton on the sulfate, a conjugate base of a strong acid, dictates the specificity of each receptor. The ability of the receptors to differentiate each oxyanion by way of the presence or absence of proton(s) is an extremely high level of sophistication in molecular recognition. We have shown by atomic structure analysis that, whereas the binding site in the phosphate receptor is designed to recognize the proton(s) of the weak acid phosphate (monobasic or dibasic form), the site in the sulfate-binding protein is designed to bind fully ionized sulfate.

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